Iranian Journal of Basic Medical Sciences

www.mums.ac.ir/basic_medical/en/index

IJBMS

Gene Expression Status and Methylation Pattern in Promoter of P15INK4b and P16INK4a in Cord Blood CD34+ Stem Cells

Mehdi Azad [1], Saeid Kaviani [1]*, Mehrdad Noruzinia [1], Yousef Mortazavi [2], Naser Mobarra [3,7], Shaban Alizadeh [4], Mohammad Shahjahani [1], Fatemeh Skandari [1], Mohammad Hosein Ahmadi [5], Amir Atashi [1], Saeid Abroun [1], Zahra Zonoubi [6]

[1]Hematology Department, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[2] Hematology Department, Zanjan Medical Sciences University, Zanjan, Iran
[3] Department of Clinical Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[4]Department of Hematology, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran
[5] Iranian Blood Transfusion Organizations, Medical Department
[6]Department of Obstetrics and Gynecology, Mahdiyeh Hospital, Shahid Beheshti University,Tehran, Iran
[7]Students' Scientific Research Center, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article type:
Original article

Article history:
Received: Aug 15, 2012
Accepted: Dec 8, 2012

Keywords:
Gene expression
Hematopoietic stem cells
Methylation
Tumor suppressor genes

ABSTRACT

Objective(s): Stem cell differentiation into different cell lineages depends upon several factors, cell cycle control elements and intracellular signaling elements, including P15INK4b and P16INK4a genes. Epigenetics may be regarded as a control mechanism which is affected by these factors with respect to their promoter structure.

Materials and Methods: The CD34 + cord blood stem cells were purified, isolated and then expanded. The undifferentiated day genome was isolated from part of the cultured cells, and the seventh day differentiated genome was isolated from the other part after differentiation to erythroid lineage. The procedure was followed by a separate Real-Time PCR for the two genes using the obtained cDNA. The processed DNA of the former stages was used for MSP (Methylation Specific PCR) reaction. Finally, pre- and post differentiation results were compared.

Results: After performing MSP for each gene, it became clear that P15INK4b gene has undergone methylation and expression in predifferentiation stage. In addition, its status has not been changed after differentiation. P15INK4b gene expression was reduced after the differentiation. The other gene, P16INK4a, showed no predifferentiation methylation. Itwas completely expressed methylated and underwent reduced expression after differentiation.

***Conclusion*:** Specific predifferentiation expression of P15INK4b and P16INK4a genes along with reduction in their expression after erythroid differentiation indicated animportant role for these two genes in biology of CD34+ cells in primary stages and before differentiation. In addition, both genes are capable of epigenetic modifications due to the structure of their promoters.

►*Please cite this paper as:*
Azad M, Kaviani S, Noruzinia M, Mortazavi Y, Mobarra N, Alizadeh Sh, Shahjahani M, Skandari F, Ahmadi MH, Atashi A, Abroun S, Zonoubi Z. Gene Expression Status and Methylation Pattern in Promoter of P15INK4b and P16INK4a in Cord Blood CD 34+ Stem Cells. Iran J Basic Med Sci; 2013; 16: 822-828.

Introduction

Investigations about the formation mechanisms of different cell types *in vitro* has led to understanding of the general mechanisms of transcription and regulation of the gene expression (1-3). In fact, differentiation process in primitive cells depends on the control of gene expression and precise regulation of the intracellular signaling. In this context, the requirement of specific control factors such as cytokines, specific transcription factors and cell cycle control elements is established and may be expected (4-8). Two of these factors classified as tumor suppressor proteins include cyclin-dependent kinase inhibitors of 2A and 2B types, respectively, known as P16INK4a and P15INK4b. P16INK4a or P16 is a tumor suppressor protein and cell cycle control element. A study by Minami *et al* (2003) highlighted the role of P16 in hematopoiesis. They showed that this gene was able to induce differentiation and apoptosis in erythroid lineage (9). CDK-4 and CDK6 are potentially inhibited by P16, therefore, the mdm 2 is not activated, and P53 protein (degraded in normal conditions by mdm 2) remains intact in these conditions and may suppress the activity of tumors (9-12). The genes related to P15 and P16 are

*Corresponding author: Saeid Kaviani. Hematology Department, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran. Tel: +98-21-82883832; Fax: +98-21-88013030; email: kavianis@modares.ac.ir